

Sulfonylurea-Mediated Stimulation of Insulin Exocytosis via an ATP-Sensitive K⁺ Channel–Independent Action

Erik Renström,¹ Sebastian Barg,¹ Frank Thévenod,² and Patrik Rorsman¹

Several reports indicate that hypoglycemic sulfonylureas augment Ca²⁺-dependent insulin secretion via mechanisms other than inhibition of the ATP-sensitive K⁺ channel. The effect involves a 65-kd protein in the granule membrane and culminates in intragranular acidification. Lowering of granule pH is necessary for the insulin granule to gain release competence. Proton pumping into the granule is driven by a v-type H⁺-ATPase, but requires simultaneous Cl⁻ uptake into the granule via metabolically regulated CIC-3 Cl⁻ channels to maintain electroneutrality. Here we discuss the possibility that modulation of granule CIC-3 channels represents the mechanism whereby sulfonylureas directly potentiate the β-cell exocytotic machinery. *Diabetes* 51 (Suppl. 1):S33–S36, 2002

Hypoglycemic sulfonylureas (SUs) elicit insulin secretion by binding to the high-affinity β-cell sulfonylurea receptor (SUR)-1 that is part of the ATP-sensitive K⁺ channel (K_{ATP}-channel), with resultant β-cell depolarization and initiation of electrical activity. Several studies suggest that SU-binding is not confined to SUR-1. [³H]-glimepiride binding to a 65-kd protein has been demonstrated (1), and Ozanne et al. (2) reported that SU-binding predominantly occurs on intracellular membranes including those of the secretory granules. These data suggest that SU may have additional effects other than K_{ATP}-channel inhibition. Indeed, we have previously demonstrated that hypoglycemic SUs also potentiate Ca²⁺-induced exocytosis in voltage-clamped β-cells (3). Although this report has caused some controversy (4), similar results have been reported in β-cells (5) and in catecholamine-secreting chromaffin cells (6). The SU-mediated potentiation of exocytosis occurs by a mechanism that involves Cl⁻ fluxes into the granule and a 65-kd protein in the granule membrane, which appears to be related to the multidrug resistance P-glycoprotein (mdr1) (7). Here we discuss in closer detail how the SUs interfere with the β-cell exocytotic machinery and the mechanism

underlying SU-mediated potentiation of Ca²⁺-dependent insulin release.

ATP-dependent priming in the β-cell. The priming reaction in exocytosis is a fundamental event in the fate of the secretory granule. Although the details are not fully elucidated, it seems clear that it involves pairing of soluble *N*-ethylmaleimide sensitive fusion protein attachment protein receptor (SNARE) proteins on the vesicles and in the target membrane and ATP-hydrolysis. Insulin secretion is indeed highly dependent on ATP (8), but the exact nature of the priming reaction in pancreatic β-cells remains elusive. Different priming enzymes have been proposed such as *N*-ethylmaleimide sensitive factor (NSF) (9,10) and phosphatidylinositol-4-phosphate 5-kinase (PtdInsP5K) (11). The role of NSF in insulin secretion (12), and secretion in general, is debated, and the enzyme is currently regarded as playing more a role in postfusion disassembly of the SNARE complex (13). Recently, we have identified the v-type H⁺-ATPase in the granule membrane as one candidate priming enzyme (14). This ATPase is responsible for generation of the acidific granule interior in mature insulin granules. The importance of a low intragranular pH for processing of the prohormone is established (15). However, that intragranular pH also affects the release competence of the insulin granule was not previously known. H⁺ pumping into the granule requires simultaneous Cl⁻ uptake via CIC-3 Cl⁻ channels to maintain electroneutrality, reminiscent of the situation in pancreatic zymogen granules (16). Otherwise, a positive granule potential quickly develops that prevents granule acidification (17). This Cl⁻ conductance determining the rate of acidification is under metabolic control. It is inhibited by decreases in the intracellular ATP/ADP ratio, and under such circumstances, granule acidification is arrested. The involvement of CIC-3 channels in acidification of synaptic vesicles was recently demonstrated in CIC-3 knockout mice (18). Modulation of CIC-3 channel activity also represents the mechanism whereby SU potentiates exocytosis, as will be discussed below.

SU-mediated effects on granule pH. SU binding to a 65-kd protein in the granule fraction was demonstrated by photoaffinity labeling with [³H]-glibenclamide in normal mouse islets (Fig. 1) and in insulin-releasing RINm5F cells (not shown). The SU-mediated actions on granular CIC-3 Cl⁻ channel activity and cotransport of H⁺ over the granule membrane were explored using the standard whole-cell configuration of the patch clamp technique. The cells were perfused with an ATP-containing intracellular solution supplemented with the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (100 μmol/l). Deprotonation of the insulin granules via this artificial

From the ¹Department of Molecular and Cellular Physiology, Institute of Physiology, Lund University, Lund, Sweden; and the ²F.T. School of Biological Sciences, University of Manchester, Manchester, U.K.

Address correspondence and reprint requests to erik.renstrom@mphy.lu.se. Accepted in revised form 20 June 2001.

K_{ATP} channel, ATP-sensitive K⁺ channel; NSF, *N*-ethylmaleimide sensitive factor; SNARE, soluble *N*-ethylmaleimide sensitive fusion protein attachment protein receptor; SU, sulfonylurea; SUR, sulfonylurea receptor.

The symposium and the publication of this article have been made possible by an unrestricted educational grant from Servier, Paris.

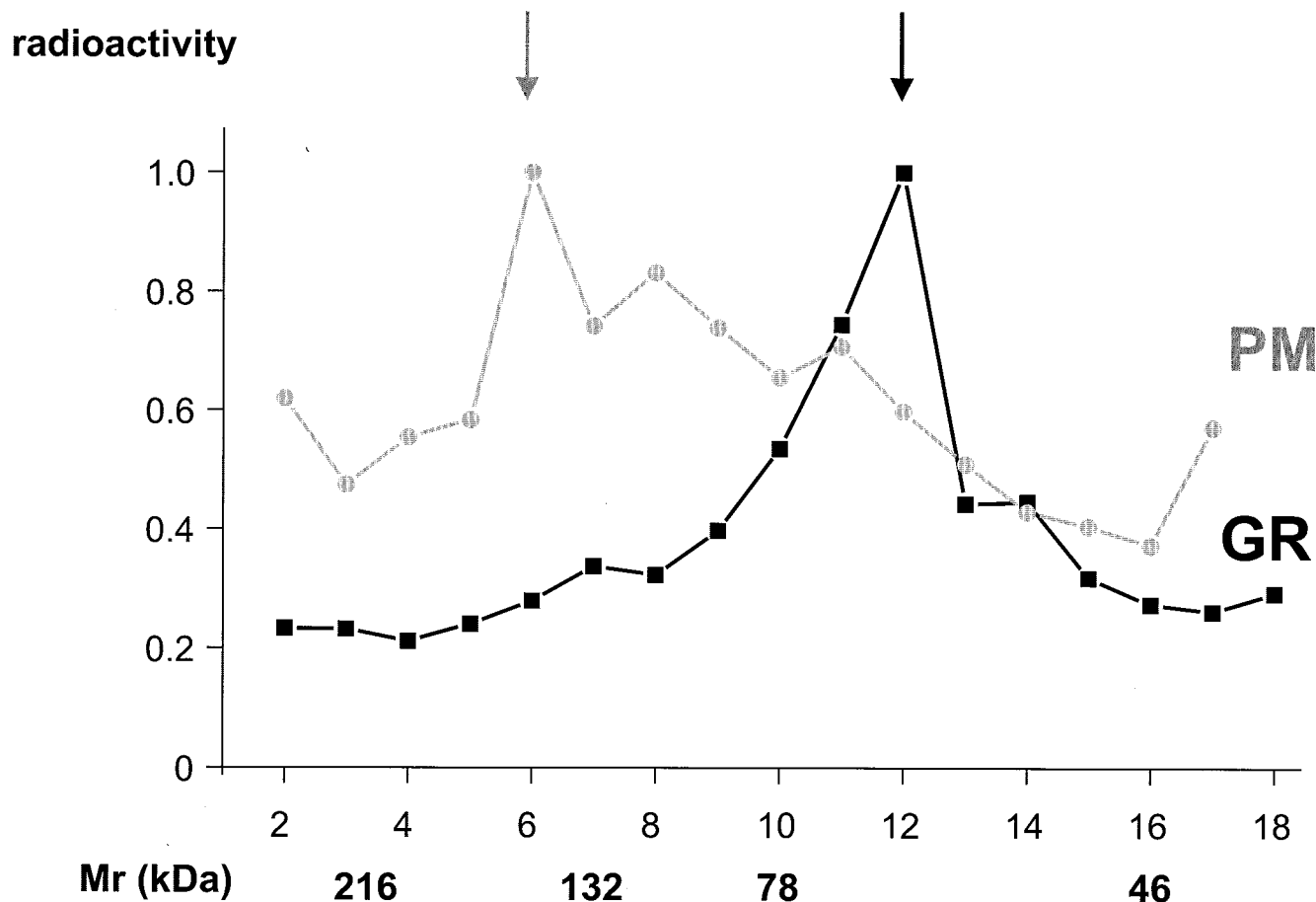


FIG. 1. Photoaffinity labeling by [^3H]-glibenclamide in the granule membrane fraction (GR; black curve) and plasma membrane fraction (PM; gray curve) of normal mouse islets. In the granule membrane fraction, a 65-kd protein is labeled, whereas in the plasma membrane fraction, a 140-kd protein with the expected molecular weight (Mr) for SUR-1 was detected. Radioactivity is plotted as fraction of peak radioactivity (599 cpm for PM and 1,109 cpm for GR). Granule protein (60 μg) and plasma membrane protein (73 μg) were equilibrated with 100 nmol/l [^3H]-glibenclamide for 1 h and ultraviolet irradiated (3 min, 312 nm). Membrane protein from granule or plasma membrane fractions were separated by SDS-PAGE on 7.5% acrylamide gels, and radioactivity measured in a liquid scintillation counter. Using a low concentration of [^3H]-glibenclamide (20 nmol/l), the granular 65-kd sulfonylurea receptor was inconsistently labeled, suggesting that it has a lower affinity than that of SUR-1. These experiments were performed using methods described in Braun et al. (19).

pathway was monitored as a rapid $\sim 60\%$ decrease in LysoSensor fluorescence 1 min after establishment of the standard whole-cell configuration (Fig. 2A–B). Deprotonation did not occur when the Cl $^-$ conductance was inhibited by the broad Cl $^-$ channel blocker 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) (100 $\mu\text{mol/l}$) or a functional antibody against the ClC-3 channel. Similar effects were observed when the cytosolic ATP/ADP ratio was decreased (3 mmol/l Mg-ATP/5 mmol/l Mg-ADP) or when the K_{ATP} channel activator diazoxide (200 $\mu\text{mol/l}$) was added to the intracellular solution. Interestingly, the inhibitory action of Mg-ADP on granule deprotonation was completely counteracted by tolbutamide (100 $\mu\text{mol/l}$). Admittedly, this assay measures H $^+$ flux in the direction opposite to that expected under physiological conditions. A major advantage is the direct demonstration of the necessity of a counterconductance for H $^+$ translocation over the granule membrane, apart from a favorable signal-noise ratio. However, it was also possible to detect the effects of Mg-ATP, Mg-ADP, and tolbutamide on H $^+$ transport into the granule using a similar LysoSensor-based assay without CCCP (14).

The most exciting observation was that cytosolic conditions that permitted rapid H $^+$ transport over the granule membrane in either of the LysoSensor assays were associated with a high exocytotic capacity (Fig. 2C–D). When the cells were dialyzed with a control Ca $^{2+}$ -containing intracellular solution (free [Ca $^{2+}$] $_i \sim 1.5 \mu\text{mol/l}$), supplemented with Mg-ATP (3 mmol/l) and cAMP (0.1 mmol/l), exocytosis was rapidly initiated after establishing the standard whole-cell condition and averaged $32 \pm 5 \text{ fF/s}$ ($n = 26$). The addition of Mg-ADP (5 mmol/l) or diazoxide (200 $\mu\text{mol/l}$) reduced exocytotic rates by 75 and 47%, respectively. Of particular interest was the fact that tolbutamide (100 $\mu\text{mol/l}$) counteracted the Mg-ADP-induced inhibition of exocytosis, and in the simultaneous presence of Mg-ADP and tolbutamide, exocytosis averaged 95% of that observed under control conditions. Thus, the effects of tolbutamide on Cl $^-$ transport/granule acidification mirror those on exocytosis.

Model for the K_{ATP} -independent actions of sulfonylureas. In Fig. 3, we have summarized our current ideas on how SUs interfere with the β -cell exocytotic machinery. SU binding to the granular 65-kd receptor protein results

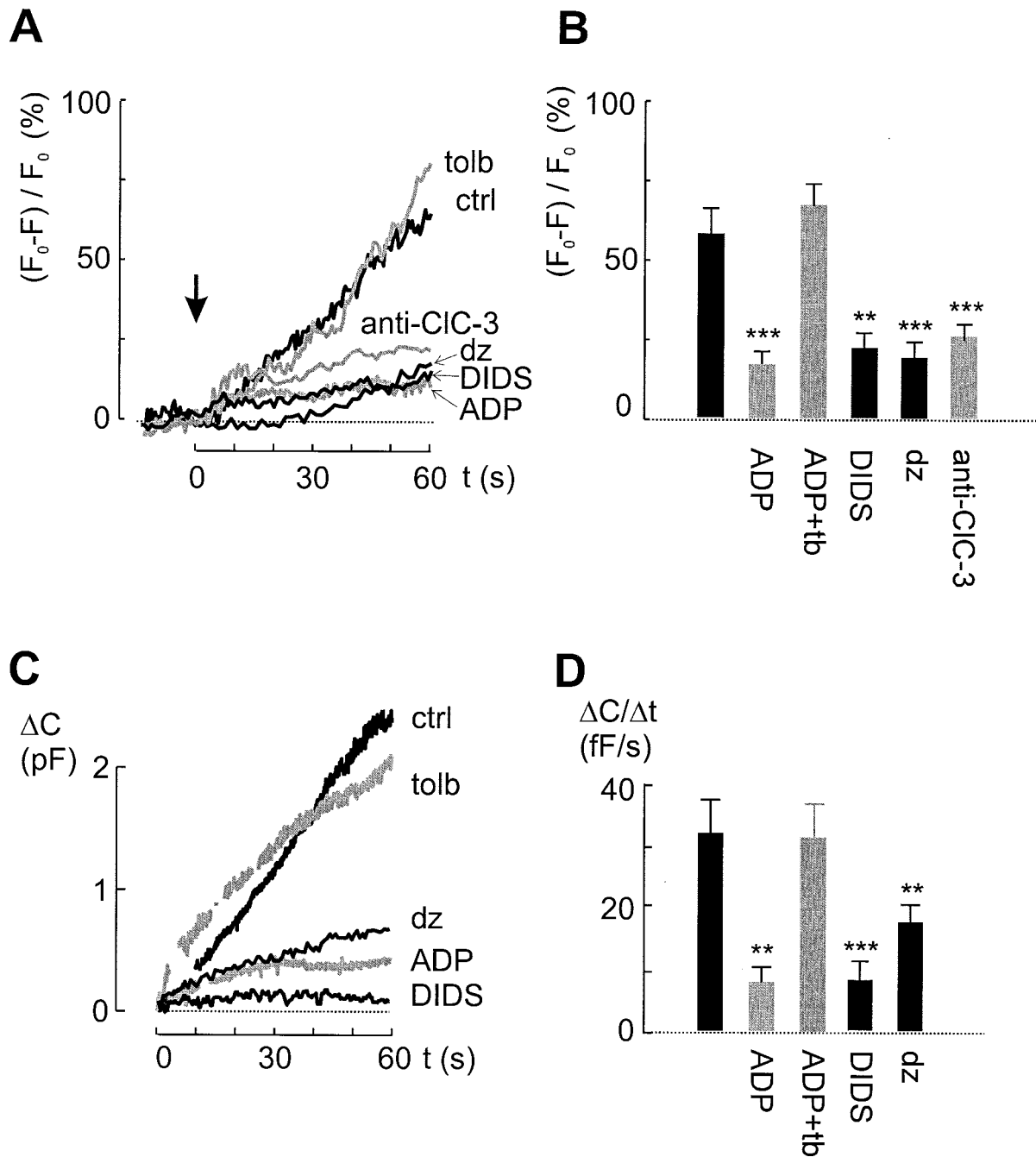


FIG. 2. *A:* Deprotonation of the granule interior estimated as the relative decrease (in percentage terms) of the initial LysoSensor-fluorescence intensity $[(F_0 - F)/F_0]$ after establishment of the standard whole-cell configuration. A standard Ca^{2+} - and ATP-containing intracellular solution was used and supplemented with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (ctrl; black, $n = 14$); ADP and CCCP (ADP; gray, $n = 9$); tolbutamide, ADP, and CCCP (tolb; gray, $n = 10$); diazoxide and CCCP (dz; black, $n = 7$); 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and CCCP (DIDS; black, $n = 5$); and anti-CIC-3, a functional antibody directed against the CIC-3 channel, and CCCP (anti-CIC-3; gray, $n = 9$). The arrow indicates the establishment of the standard whole-cell configuration. *B:* Average decrease (percentage of initial) in LysoSensor fluorescence after 60 s recording $[(F_0 - F)/F_0]$. Data are means \pm SE. Statistical significances were evaluated comparing the responses in the respective groups with the responses obtained with the control solution including CCCP. *C:* Increases in cell capacitance (ΔC) under control conditions (ctrl; black, $n = 26$), after addition of Mg-ADP (ADP; gray, $n = 12$), tolbutamide and Mg-ADP (tolb; gray, $n = 12$), diazoxide (dz; black, $n = 19$), or DIDS (black, $n = 8$). *D:* Average rate of changes in cell capacitance ($\Delta C/\Delta t$) \pm SE. ** $P < 0.01$; *** $P < 0.001$. Data are modified from Barg et al. (14), where the methodology is described in detail.

in increased granular CIC-3 channel activity. In analogy with the situation in the K_{ATP} channel (20), it can be hypothesized that ATP directly interacts with the CIC-3 channel, whereas ADP binds to the granular SU receptor. In this scenario, SU binding would then relieve the ADP-

mediated effects, and thereby accelerate acidification of the granule interior and priming for exocytosis. Future work is required to establish the exact identity of the granular SU receptor and its interactions with the CIC-3 channels.

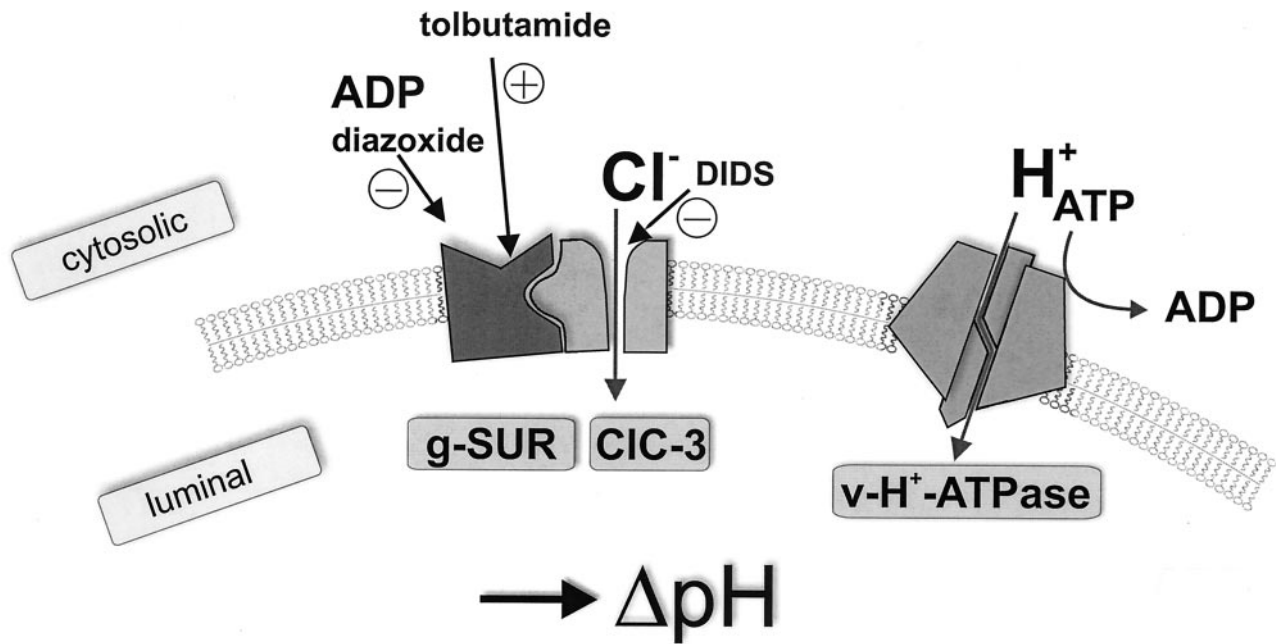


FIG. 3. Model for the sulfonylurea-mediated stimulation of Ca^{2+} -dependent insulin secretion. Sulfonylurea binding to a 65-kd receptor in the granule membrane (g-SUR) activates granular CIC-3 Cl^- channels (CIC-3). The CIC-3 channels act in concert with the v-type H^+ -ATPase in the granule membrane to promote acidification of the granule interior, which is essential for the insulin granule to gain release competence. The exact nature of the interaction between g-SUR and the CIC-3 channel remains to be established. This putative g-SUR/CIC-3 channel complex is, however, under metabolic control and is activated when the ATP/ADP ratio is high. By analogy to the K_{ATP} channel, it seems likely that the inhibitory actions of ADP and diazoxide are mediated via g-SUR, whereas 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) acts directly on the CIC-3 channel. The sulfonylurea-mediated stimulation of granule acidification, and in turn exocytosis, then result from perturbation of the ADP-mediated actions.

ACKNOWLEDGMENTS

We thank our colleagues in Lund and Manchester for sharing unpublished data. Financial support was obtained from the Swedish Medical Research Council (grants 8647, 9890, 12334, 12708, and 13147), the Swedish Diabetes Association, the Royal Physiographic Society, the Juvenile Diabetes Foundation International, the Knut and Alice Wallenbergs Stiftelse, the European Community, and the Novo Nordisk Foundation and DFG Th 345/6-1 to F.T.

REFERENCES

- Kramer W, Muller G, Geisen K: Characterization of the molecular mode of action of the sulfonylurea, glibenclamide, at beta-cells. *Horm Metab Res* 28:464–468, 1996
- Ozanne SE, Guest PC, Hutton JC, Hales CN: Intracellular localization and molecular heterogeneity of the sulphonylurea receptor in insulin-secreting cells. *Diabetologia* 38:277–282, 1995
- Eliasson L, Renström E, Åmmälä C, Berggren PO, Bertorello AM, Bokvist K, Chibalin A, Deeney JT, Flatt PR, Gäbel J, Gromada J, Larsson O, Lindström P, Rhodes CJ, Rorsman P: PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic β cells. *Science* 271:813–815, 1996
- Mariot P, Gilon P, Nenquin M, Henquin JC: Tolbutamide and diazoxide influence insulin secretion by changing the concentration but not the action of cytoplasmic Ca^{2+} in beta-cells. *Diabetes* 47:365–373, 1998
- Tian YA, Johnson G, Ashcroft SJ: Sulfonylureas enhance exocytosis from pancreatic β -cells by a mechanism that does not involve direct activation of protein kinase C. *Diabetes* 47:1722–1726, 1998
- Taylor SC, Carpenter E, Roberts ML, Peers C: Potentiation of quantal catecholamine secretion by glibenclamide: evidence for a novel role of sulphonylurea receptors in regulating the Ca^{2+} sensitivity of exocytosis. *J Neurosci* 19:5741–5749, 1999
- Barg S, Renström E, Berggren PO, Bertorello A, Bokvist K, Braun M, Eliasson L, Holmes WE, Köhler M, Rorsman P, Thévenod F: The stimulatory action of tolbutamide on Ca^{2+} -dependent exocytosis in pancreatic β cells is mediated by a 65-kd mdr-like P-glycoprotein. *Proc Natl Acad Sci U S A* 96:5539–5544, 1999
- Eliasson L, Renström E, Ding WG, Proks P, Rorsman P: Rapid ATP-dependent priming of secretory granules precedes Ca^{2+} -induced exocytosis in mouse pancreatic β -cells. *J Physiol* 503:399–412, 1997
- Hanson PI, Heuser JE, Jahn R: Neurotransmitter release: four years of SNARE complexes. *Curr Opin Neurobiol* 7:310–315, 1997
- Sutton RB, Fasshauer D, Jahn R, Brünger AT: Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature* 395:347–353, 1998
- Hay J, Fiset PL, Jenkins GH, Fukami K, Takenawa T, Anderson RA, Martin TF: ATP-dependent inositolide phosphorylation required for Ca^{2+} -activated secretion. *Nature* 374:173–177, 1995
- Kiraly-Borri CE, Morgan A, Burgoyne RD, Weller U, Wollheim CB, Lang J: Soluble N-ethylmaleimide-sensitive-factor attachment protein and N-ethylmaleimide-insensitive factors are required for Ca^{2+} -stimulated exocytosis of insulin. *Biochem J* 314:199–203, 1996
- Weber T, Parlati F, McNew JA, Johnston RJ, Westermann B, Sollner TH, Rothman JE: SNAREpins are functionally resistant to disruption by NSF and alphaSNAP. *J Cell Biol* 149:1063–1072, 2000
- Barg S, Huang P, Eliasson L, Nelson DJ, Obermüller S, Rorsman P, Thévenod F, Renström E: Priming of insulin granules for exocytosis by granular chloride uptake and acidification. *J Cell Sci*. In press
- Hutton JC: The insulin secretory granule. *Diabetologia* 32:271–281, 1989
- Thévenod F, Anderie I, Schulz I: Monoclonal antibodies against MDR1 P-glycoprotein inhibit chloride conductance and label a 65 kd protein in pancreatic zymogen granule membranes. *J Biol Chem* 269:24410–24417, 1994
- al Awqati Q, Barasch J, Landry D: Chloride channels of intracellular organelles and their potential role in cystic fibrosis. *J Exp Biol* 172:245–266, 1992
- Stobrawa SM, Breiderhoff T, Takamori S, Engel D, Schweizer M, Zdebik AA, Bosl MR, Ruether K, Jahn H, Draguhn A, Jahn R, Jentsch TJ: Disruption of CIC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. *Neuron* 29:185–196, 2001
- Braun M, Anderie I, Thévenod F: Evidence for a 65 kD sulphonylurea receptor in rat pancreatic zymogen granule membranes. *FEBS Lett* 411: 255–259, 1997
- Ashcroft FM, Gribble FM: New windows on the mechanism of action of $\text{K}(\text{ATP})$ channel openers. *Trends Pharmacol Sci* 21:439–445, 2000